REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-00-

0257

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE

3. REPORT TYPE AND DATES COVERED
FINAL -15 Nov 97 - 30 Apr 99

4. TITLE AND SUBTITLE
Dermal Absorption of Chemicals From Evaporating Vehicles

5. FUNDING NUMBERS
F49620-98-1-0060

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
802 N Randolph St., Rm 732
Arlington VA 22203-1977

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for Public Release: Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
Unfortunately, only year 01 of this project was funded. Initial work on the project progressed slower than schedule due to delays in finding a graduate student to work on the project and in delivery of key instruments (i.e., the gas chromatograph and diffusion cell console). Consequently, work continued beyond the end of Year 01 (November 14, 1998) until April 30, 1999 under a no-cost extension. Significant progress has been made towards the principal research objective of the project. As originally proposed, this 3-year study would have included experimental measurements (i) skin-partition coefficients and (ii) dermal absorption from vapors, occluded vehicles and non-occluded volatile vehicles), combined with mathermatical models for interpreting and extrapolating the experimental results to other chemicals. During the first and only year of this project, many experimental measurements were completed, and, and initial mathematical models were developed.

14. SUBJECT TERMS
Chromatograph, Diffusion, vapors

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASS	UNCLASS	UNCLASS	

Sandy,
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1. Cover Page

FINAL REPORT

August 3, 1999

Bioenvironmental Science Grant Program

WJK

P.S. Could I have a copy of this?

Grant Number:	**F49620-98-1-0060**
Project Title:	**Dermal Absorption of Chemicals from Evaporating Vehicle Mixtures**
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2. Objective

The objective of this project is to develop procedures for estimating dermal absorption of organic chemicals from evaporating vehicles (such as JP8) using absorption data from occluded (nonevaporating) aqueous solutions. To achieve this goal requires two related but separate studies. First, the relationship between dermal absorption measurements from nonaqueous and aqueous vehicles will be established when evaporation is prevented. Second, the theoretical description of the evaporation process will be experimentally demonstrated. The research approach uses mathematical models of the evaporation and absorption processes to design, and then to analyze data from, dermal absorption experiments, and to address the 5 specific aims of the project, namely: (i) to test if lipophilic vehicles enhance the rate or extent of dermal absorption of other chemicals; (ii) to determine the cause and to assess predictability when vehicles do enhance the rate or extent of dermal absorption of other chemicals; (iii) to test if dermal absorption parameters, namely permeability coefficients and partition coefficients, from occluded nonaqueous vehicles are related predictably to parameters measured (or estimated from measurements made) from occluded aqueous vehicles; (iv) to test if the changes in contact area and concentrations which occur when the vehicle evaporates are predictable and if these changes affect predictably the amount and rate of dermal absorption; and (v) to continue development of skin property databases.

3. Accomplishments and Status of Effort

Unfortunately, only Year 01 of this project was funded. Initial work on the project progressed slower than schedule due to delays in finding a graduate student to work on the project and in delivery of key instruments (i.e., the gas chromatograph and diffusion cell console). Consequently, work continued beyond the end of Year 01 (November 14, 1998) until April 30, 1999 under a no-cost extension.

Significant progress has been made towards the principal research objective of the project. As originally proposed, this 3-year study would have included <u>experimental measurements</u> [(i) skin-partition coefficients and (ii) dermal absorption from vapors, occluded vehicles and non-occluded volatile vehicles], combined with <u>mathematical models</u> for interpreting and extrapolating the experimental results to other chemicals. During the first and only year of this project, many experimental measurements were completed, and initial mathematical models were developed. The more significant results are described next.

<u>Experimental Measurements.</u> A gas chromatograph was acquired, set-up and analytical procedures developed for several of the chemicals in this study (i.e., toluene, hexane, naphthalene, and 4-cyanophenol). A diffusion cell apparatus was acquired and preliminary experiments conducted to demonstrate procedures for operating, and data analysis.

A source of rat skin was established and regular skin acquisition begun. Because we do not have an animal care facility at CSM, there are many advantages in using previously frozen tissues. Although dermal absorption from fresh and frozen human skin has been showed to be similar, this has never been demonstrated for rat skin. Consequently, we conducted a series of experiments comparing chemical penetration through rat skin that was (1) freshly acquired, (2) stored on culture media and used within 24 hours, and (3) frozen. The experiments were designed so that skin from each rat was used in all three scenarios. The results from experiments with saturated aqueous solutions of 4-cyanophenol (n = 12 rats) indicate that there is no statistical

difference between the three skin handling methods. Consequently, frozen rat skin was used in most of the subsequent experiments.

All rat skin samples were taken from the back following procedures similar to those used by Dr. Jim McDougal at Wright-Patterson Air Force Base. The back of each animal is large enough to provide skin for 3 diffusion cell experiments. The region of the back (i.e., the front, middle or rear) from which each sample is taken is noted. For the Sprague Dawley rats used in our study, the statistical variations between body region (i.e., the front, middle or rear of the animal's back) were not statistically significant. This fact, combined with the use of frozen skin, made it possible to use a series of statistically designed studies that account for animal-to-animal variability in comparisons of various dosing regimens. For example, we have used the Balanced Incomplete Block Design (specifically, a doubled 7 x 7 Youden Square) shown in Table 1 to examine the penetration rate of 7 different doses of a solvent deposited test chemical (in this case, 4-cyanophenol delivered in acetone). Six diffusion cell experiments were conducted for each dose. The skin for the entire study, 14 animals, was collected in a two-hour period, frozen and then used as specified in Table 1. The entire investigation of the 7 different dose regimens took about 10 weeks. To confirm that the period of freezing has not affected the measurements, in future measurements, we will require that Dose 7 (i.e., the experiments using skin frozen the longest time) replicates Dose 1 (i.e., the experiments using skin frozen the shortest time).

Table 1

Experimental Design for Studying Various Dosing Regimens

Animal No.	Dose Regimen No.						
	1	2	3	4	5	6	7
1	F*	M	R				
2	M			R	F		
3	R	R				F	M
4		F		F	M		
5						M	R
6			F	M	R		
7	F		M			R	F
8	M		R				
9	R			R	F		
10						F	M
11		R		F	M		
12		F				M	R
13			F	M	R		
14			M			R	F

*location of the skin sample is noted as F = front, M = middle and R = rear

Dermal absorption measurements have been completed for naphthalene from water and for 4-cyanophenol from water, acetone, propylene glycol and as a pure solid powder. This is the first step in developing a procedure for using aqueous penetration data to estimate absorption from volatile, non-aqueous solvents. Several of these experiments were designed to examine differences in dermal absorption from saturated aqueous solutions compared to the pure chemical, either placed on skin as a powder or deposited from a volatile solvent like acetone. Because skin penetration proceeds through a solution-diffusion mechanism, penetration rates are directly related to thermodynamic activity of the chemical in contact with the skin surface. Since thermodynamic activity of a saturated aqueous solution is the same as for a pure solid of the same chemical, differences in penetration rates indicate differences in the mass transfer resistances at the skin-vehicle interface or in vehicle effects on skin permeability.

In diffusion cell experiments of saturated aqueous solutions of naphthalene, the penetration rates were larger than observed by Dr. James McDougal for pure powdered naphthalene. This is consistent with the decreased ability of the solid naphthalene particles to partition into the skin compared to the aqueous solution. In addition, as expected, the penetration rate for the more volatile solid naphthalene was larger than from the less volatile solid 4-cyanophenol. These results are relevant to exposure to chemical residues left on skin after the more volatile components from fuels have evaporated.

Like naphthalene, pure 4-cyanophenol penetrated skin more slowly from pure powder than from a saturated aqueous solution. However, when 4-cyanophenol solid was deposited onto the skin surface using acetone, the penetration rates were comparable to saturated aqueous solutions as long as the amount applied was sufficient to cover the entire exposed area. When the doses of 4-cyanophenol applied in acetone were too small to completely cover the entire area, then the penetration rates were less than from a saturated aqueous solution.

Mathematical Models. As part of an earlier research project funded through the Air Force Office of Scientific Research (see publications included with this report), we demonstrated that it is possible to represent a membrane process like dermal absorption by using a carefully crafted well-stirred compartment (i.e., pharmacokinetic) models. (We have also showed that the commonly used compartment models can represent membrane absorption dynamics poorly.) We have used these results as the starting place in this project for developing models that include penetration and evaporation of all vehicle constituents. Preliminary models are now completed for a non-penetrating, evaporating vehicle containing a penetrating, but non-evaporating chemical.

In addition to these pharmacokinetic well-stirred compartment models, we have also developed two other models that describe dermal absorption of chemicals deposited using volatile solvents. Model One was designed to represent dermal absorption from deposited chemical films that do and do not completely cover the exposed skin surface. This model does account for changes in the amount remaining on the skin surface as chemical is dermally absorbed. However, Model One only considers diffusion across the stratum corneum perpendicular to the skin surface. Model Two includes contributions of the viable epidermis and diffusion horizontal to the skin surface in both the viable epidermis and stratum corneum. However, Model Two does not adjust the calculations for the amount remaining on the skin surface as dermal absorption occurs. Thus, Model Two should only be used to represent dermal absorption data for situations in which the absorbed fraction of the amount applied is small (< about 20%).

The results of Model One have been applied to data reported by G. Kasting [AAPS, 1997, Boston, MA & *Pharm.Res.*, S313, 1997]. In these experiments, dermal penetration of a model

permeant, vanillylnonanamide (VN, molecular weight = 293, log $K_{o/w}$ = 3.74)), was measured across excised human skin over 72 hours from 8 applied doses (i.e., 1.4 to 4,428 μg VN/cm^2). Doses of VN were applied to the skin surface in small amounts of propylene glycol (i.e., 6 – 25 μL/cm^2). Figure 1 compares model calculations with the Kasting data, where $M_{sc,out}$ designates the cumulative mass appearing in the receptor chamber of the diffusion cells. Each data point in Figure 1 represents the mean value of 7 measurements. With only three adjustable parameters, Model One reasonably characterizes the measurements from all 8 doses over 72 hours. Based on these parameters, the amount of VN required to completely cover the skin is at least 120 μg/cm^2 and the maximum flux when the exposed skin is entirely covered with a film of VN is 2.42 μg/cm^2/hr.

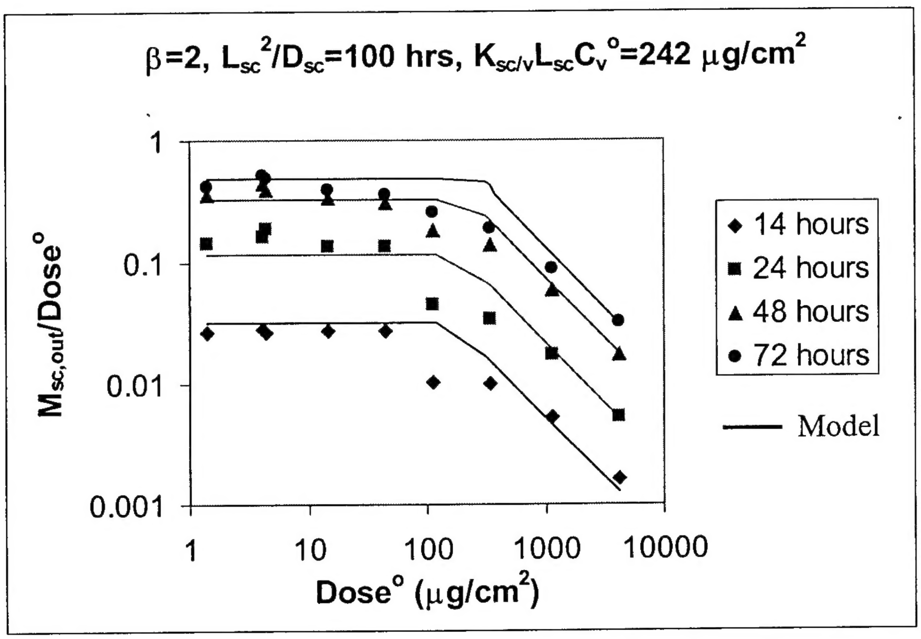

Figure 1 – Comparison of experimental data and model calculations of the fraction of applied dose penetrating skin plotted as a function of applied dose.

4. Personnel Supported

Colorado School of Mines

Annette L. Bunge — Professor. *Principal Investigator*

Lixin Sun — Graduate Research Associate

Karin Stephens — Undergraduate Research Assistant (skin absorption database assistant)

Note that three graduate students (specifically, Lixin Sun, Kelly McCarley and Micaela Reddy) have worked as a team on different aspects of three funded projects. However, to minimize accounting paperwork, financial support of each student was assigned to an individual project. Consequently, Kelly McCarley and Micaela Reddy have both worked on mathematical models for this project, although they were not financially supported on the project.

Centre Interuniversitaire de Recherche et d'Enseignement

Richard H. Guy Professor. *Co-Principal Investigator*

5. Publications

No results from this project have been published yet. However, two manuscripts describing the mathematical models are nearly complete and should be submitted for publication in the next few months. The experimental data collected as part of this project are insufficient for a single paper, but will eventually be published in combination with experimental results collected as part of other projects. Tentative titles for the nearly completed manuscripts are:

K.D. McCarley and A.L. Bunge, Dermal Absorption of Solvent Deposited Chemicals, in preparation.

M.B. Reddy and A.L. Bunge, A two-dimensional mathematical model of dermal absorption of chemicals distributed on the skin surface, in preparation.

On a related note, we are continuing to publish papers based on results from an earlier AFOSR project (i.e., F49620-95-1-021). These are listed below. I have included a reprint with this report of the one paper published earlier this year. Reprints from papers published in 1998 or earlier have already been sent. We will continue to send reprints as publication occurs.

A.L. Bunge and J.N. McDougal, Dermal uptake, Ch. 6, In: Exposure to Contaminants in Drinking Water, Ed. S.S. Olin, CRC Press, Boca Raton (1999).

A.L. Bunge et al., On the determination of a diffusional pathlength through the stratum corneum, Intl. J. Pharmaceutics, in press.

K.D. McCarley and A.L. Bunge, Physiologically relevant two compartment pharmacokinetic models for skin, J. Pharm. Sci., accepted.

K.D. McCarley and A.L. Bunge, Pharmacokinetic models for skin—A review, J. Pharm. Sci., submitted.

K.D. McCarley and A.L. Bunge, Comparing one and two-compartment physiologically relevant models of skin, J. Pharm. Sci., submitted.

J.J. Hostynek, R.S. Hinz, C.R. Lorence and R.H. Guy, Human Skin Penetration by Metal Compounds. Chapter in Dermal Absorption and Toxicity Assessment, pp. 647-668. Edited by M.S. Roberts and K.A. Walters, New York: Marcel Dekker, 1999.

A. Naik, Y.N. Kalia, F. Pirot and R.H. Guy, Characterization of Molecular Transport Across Human Stratum Corneum In Vivo. Chapter in Percutaneous Absorption, 3rd Edition, pp. 149-175. Edited by R.L. Bronaugh and H.I. Maibach. New York: Marcel Dekker, 1999.

R.H. Guy, J.J. Hostynek, R.S. Hinz, and C. Lorence, Metals and the Skin - Topical Effects and Percutaneous Absorption. New York: Marcel Dekker, 1999.

6. Interactions/Transitions

(a) Conferences, etc.

6th International Conference on Contaminated Soil, Edinburgh, United Kingdom, May 17-21, 1998.

S.M. Arnold et al. Release kinetics of 4-cyanophenol from soil into cellulosic membranes.

Dermal Exposure Workshop. US Environmental Protection Agency, National Exposure Research Laboratory (NERL), Research Triangle Park, NC, September 17, 1998.

Perspectives in Percutaneous Penetration - 6th International Conference. Leiden, The Netherlands, Sept. 22-26, 1998.

R.H. Guy. Peptide delivery by iontophoresis.
G.D. Touraille et al. Uptake of 4-cyanophenol from soils, water and pure solids.

American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition, San Francisco, CA, November 15-19, 1998.

G.D. Touraille et al. Determining dermal absorption by tape stripping in vivo human stratum corneum: Experiments and Theory.
G.D. Touraille et al. In vivo and in vitro measurements of percutaneous permeability parameters.
B.E. Vecchia et al. Comparison of permeability coefficients for excised skin from humans and animals.

Society for Risk Analysis, Annual Meeting, Phoenix, AZ, Dec. 6-9, 1998.

A.L. Bunge. Effect of dose on dermal absorption from liquid and solid vehicles.

US Environmental Protection Agency, Dermal Exposure Workshop, Washington, DC, Dec. 10-11, 1998.

Gordon Research Conference on Barrier Function of Mammalian Skin, Il Ciocco, Italy, April 18-23, 1999.

Symposium on the Impact of Pharmacokinetics in Modern Drug Development, San Francisco, CA, May 17, 1998.

R.H. Guy. Skin Barrier Function and Transdermal Drug Delivery

Seminars.

A.L. Bunge. Dermal absorption of chemicals: Data analysis and prediction. University of Iowa, College of Dentistry, Iowa City, IA (April, 1998).

A.L. Bunge. Predicting dermal absorption from various media. United States Environmental Protection Agency, National Exposure Research Laboratory, Las Vegas, NV (August, 1998).

A.L. Bunge. Effect of Dose on Dermal Absorption from Liquid and Solid Vehicles, Society for Risk Analysis, Annual Meeting, Phoenix, AZ (Dec. 1998)

R.H. Guy. Prediction of percutaneous absorption. Centre International de Recherche Dermatologique, Sophia Antipolis, France (Feb. 1998).

R.H. Guy. Prediction of percutaneous absorption. Univ of Wales, Cardiff (Feb., 1998).

R.H. Guy. Mechanisms of Percutaneous Absorption. University of Lyon, France (March, 1998).

R.H. Guy. Why Doesn't Skin Leak? Department of Biopharmaceutical Sciences, University of California, San Francisco, CA (April, 1998).

R.H. Guy. Differential Scanning Calorimetry, Infrared Spectroscopy and Skin Barrier Function. Models to Predict Percutaneous Absorption. Institut de Recherche Pierre Fabre, Castanet Tolosan, France (September, 1998).

R.H. Guy. Administration Transdermique des Médicaments. 13ème Séminaire de 3ème Cycle en Sciences Pharmaceutiques, "Biodisponibilité et Ciblage des Médicaments". Zermatt, Switzerland (October, 1998).

R.H. Guy. Transdermal Delivery Technologies. UKaps Annual Conference, Manchester, England (June, 1999).

(b) Consultive and Advisory Functions

Annette L. Bunge

Scientific advisor U.S. Environmental Protection Agency, peer review of *Dermal Risk Assessment Interim Guidance, Supplemental Guidance to the Risk Assessment Guidance for Superfund.*

Member of the Working Group on the *Estimation of Dermal and Inhalation Exposures to Contaminants in Drinking Water*, organized by the International Life Sciences Institute of the Risk Science Institute through a Cooperative Agreement with the U.S. Environmental Protection Agency's Office of Water. This work has now been published in a book, *Exposure to Contaminants in Drinking Water: Estimating Uptake through the Skin and by Inhalation*, Ed. S.S. Olin.

Member of the Interagency Dosimetry Project working group developing unified methods for estimating cancer and non-cancer risks from exposure by ingestion, inhalation and dermal. U.S. Environmental Protection Agency is leading this effort.

Richard H. Guy

Member, Pharmacological Sciences Study Section, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD.

Participant, Dermal and Ophthalmic Drugs Advisory Committee, U.S. Food & Drug Administration, Rockville, MD.

(c) Transitions

There have been several important technology transitions resulting directly from this and also our earlier AFOSR funding. The most significant, both in time spent and information transferred, has been Professor Bunge's advisory role to several groups working on various aspects of risk

assessment at the U.S. Environmental Protection Agency. In addition, we have collaborated closely with Dr. James McDougal at Wright-Patterson AFB. These activities are described below.

Customer: US EPA, Interagency Dosimetry Project, A. Jarabek, Research Triangle Park, NC

Result: Providing expert opinion on methods for estimating dermal absorption to the working group preparing a document recommending a unified approach for estimating human health risk from cancer and non-cancer toxicity. Nearly all recommendations have been based on modeling calculations performed as part of this and the previous AFOSR project.

Application: The recommended strategies are being included into a procedure document.

Customer: Elaine Cohen-Hubel and Marc Rigas, National Exposure Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC and Las Vegas, NV

Result: Sharing mathematical modeling results and strategies for estimating dermal absorption from a variety of media including solvent delivered chemicals

Application: Human health risk assessment calculations, particularly with respect to the Food Quality Protection Act requirements.

Customer: Robert Zendzian, Office of Pesticide Programs, U.S. Environmental Protection Agency, Crystal City, VA

Result: Computations from the distributed chemical models (i.e., Model One and Two) for analyzing the large body of pesticide data delivered to EPA by pesticide registrants.

Application: Predicting human health risk from dermal exposure to pesticide formulations.

Customer: Kim Hoang, Region 10, U.S. Environmental Protection Agency, San Francisco, CA

Result: Methods for estimating dermal absorption from chemicals in aqueous solutions.

Application: Development of standard methodology for estimating dermal absorption from exposure to chemicals in drinking and surface waters.

Customer: James N. McDougal, Ph.D., Geo-Centers, Inc., Wright-Patterson AFB, Ohio

Result: Sharing and comparison of diffusion cell operating procedures, analysis, and results.

Application: Experimental results collected at WPAFB and CSM have been compared to demonstrate experimental reproducibility.

Customers: Dr. Robert Taalman, European Chemical Industry Council
Dr. Christopher Money, Exxon, UK
Dr. W. Rozenboom, Health Council of the Netherlands.

Result: In all three cases, contact was initiated to discuss human dermal exposure scenarios and methods to measure/predict the absorption of potentially toxic chemicals across the skin.

Application: Information was disseminated on our efforts to construct useful and practical experimental/theoretical models of dermal transport and the assembly of this information into simply accessible databases.

(d) New Discoveries, Inventions, or Patent Disclosures

The combined databases, in modified form, were demonstrated at the Gordon Research Conference on "Barrier Function of Mammalian Skin", Il Ciocco, Italy, April, 1999.